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EFFECT ON MAN OF PARENTERAL ADMINISTRATION OF A PRODUCTION-LINE MIXTURE OF ISOMERS 2 AND 4, EA 2233 (U)

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CHEMICAL RESEARCH AND DEVELOPMENT LABORATORIES
Edgewood Arsenal, Maryland 21010

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(U) FOREWORD

The work described in this memorandum was authorized under Project 1C522301A079, Non-Defense Medical Aspects of Chemical Agents (U). The experimental data are contained in notebook MN-1950. This work was started in November 1964 and completed in June 1965.

The human subjects in the tests conducted by this installation are enlisted US Army volunteers. There is no coercion or enticement to volunteer. The most stringent medical safeguards surround every human test.

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(C) EFFECT ON MAN OF PARENTERAL ADMINISTRATION OF A PRODUCTION-LINE MIXTURE OF ISOMERS 2 AND 4, EA 2233 (U)

I. (C) INTRODUCTION.

Orally, pure isomer 2 of EA 2233 ([3-(1,2-dimethylheptyl)-1-hydroxy-7,8,9,10-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyranyl] acetate) causes orthostatic hypotension in man at a dose of 5 µg/kg, and isomer 4 causes orthostatic hypotension at 10 µg/kg.* A 50:50 mixture of isomers 2 and 4 given orally produces orthostatic hypotension at a dose of approximately 10 µg/kg.** The present study was to determine the effect of a production-line mixture of isomers 2 and 4 when given intravenously.

II. (U) METHODS.

Subjects were 38 enlisted men, Medical Research Volunteers. They were brought to the ward area approximately 12 hr before receiving the compound in order to acclimate them to the environment. On the morning of the test, baseline studies were done. Blood pressure and pulse were recorded while the subjects were supine, immediately upon standing, after standing 5 min and again after returning to the supine position. After each blood pressure reading, a short lead II electrocardiogram (EKG) was taken. This routine was repeated at least 4 times in the 2 hr prior to injection of the compound, and every half hour after injection.

A baseline of 8 Number Facility test scores was obtained. Every 2 hr after injection, Number Facility scores, pupil size, and temperature were determined.

The following laboratory determinations were made on the morning of the test, at 24 hr, 48 hr, and 10 days after injection of the compound: white blood cell count, hematocrit, hemoglobin, serum glutamic oxaloacetic and pyruvic transaminases, alkaline phosphatase, bilirubin, blood urea nitrogen, and urinalysis. Total urine output over the test period was also measured.

The compound was supplied in 1-cc ampules in a 1 mg/ml concentration. The ampules were further diluted in 9 ml of propylene glycol to make a 100 µg/ml solution. The total dose given to any one volunteer did not exceed 225 µg. (Individual doses ranged from 0.5 to 2.55 µg/kg of body weight.) Therefore, the amount of fluid injected ranged from 0.37 to 2.25 ml. This amount was uniformly injected intravenously over a 30-sec to 60-sec time interval.

*(U) Rakatansky, H. Preliminary Report of a Pilot Study of Isomers 2 and 4 in Humans. In Summary of Clinical Studies on EA 2233 (U). Human Investigation Branch, 26 Feb 63, SMUCR-M. CONFIDENTIAL Document.

**(U) Rakatansky, H. EA 2233 Isomers, Summary Report (U). Memorandum for Record, SMUCR-M, 17 Feb 64. CONFIDENTIAL Document.

Four volunteers served as controls and were given saline. Subjects remained in bed throughout the test period or until free of symptoms.

III. (C) RESULTS.

(C) The most important response to the production mixture of isomers 2 and 4 of EA 2233 was orthostatic hypotension. In this report, orthostatic hypotension is defined as a difference of at least 10 mm Hg between the supine and standing (5 min) mean blood pressure readings, occurring on two successive readings taken a half an hour apart. Mean blood pressure is defined as one-third the difference between the systolic and diastolic blood pressures added to the diastolic pressure.

(U) Such symptoms as lightheadedness, nausea, and pallor were noted but were not always present. Also, a few individuals had symptoms without a drop in blood pressure. These symptoms will be mentioned, but the actual response sought and upon which the MED50 is based was the drop in mean blood pressure.

(U) The hypotensive responses are summarized in table 1. Nineteen men experienced orthostatic hypotension. None of 4 men were affected by 0.5 to 1.0 μg/kg; 1 out of 4 developed hypotension at 1.25 μg/kg; 2 out of 4 at 1.5 μg/kg; 2 out of 8 at 1.75 μg/kg; 5 out of 8 at 2.0 μg/kg; 3 out of 4 at 2.25 μg/kg; and 6 out of 6 at 2.55 μg/kg.

(U) Probit analysis by the method of Bliss* indicates that the minimal effective dose for producing orthostatic hypotension in 50 per cent of the subjects is 1.8 μg/kg, with 95% confidence limits of 1.51-2.07 μg/kg (figure 1). Figure 2 shows the blood pressure readings on a subject who had a minimal response.

(U) Most of the subjects with a positive response developed orthostatic hypotension between 1/2 and 3 hr after injection of the compound. Three subjects developed orthostatic hypotension between 3-1/2 to 6 hr after injection. The average time of onset of symptoms for all those volunteers with positive responses was 2.4 hr.

(U) The onset, severity, and duration of orthostatic hypotension tended to be dose-related. The average duration of orthostatic hypotension in this series was 9 hr. By definition the duration of orthostatic hypotension could not be less than 30 min.

(U) A severe reaction occurred in subject No. 12 (figure 2), who was given 1.5 μg/kg. From 3 to 5 hr post-injection, he became faint upon standing, his blood pressure could not be recorded, and he was unable to stand for the full 5 min. Subject No. 24 had the severest reaction in this series. He had a response that persisted for 28 hr following 2 μg/kg.

*Bliss, C. I. The Statistics of Bioassay. Academic Press, Inc., New York, 1952.

(C)

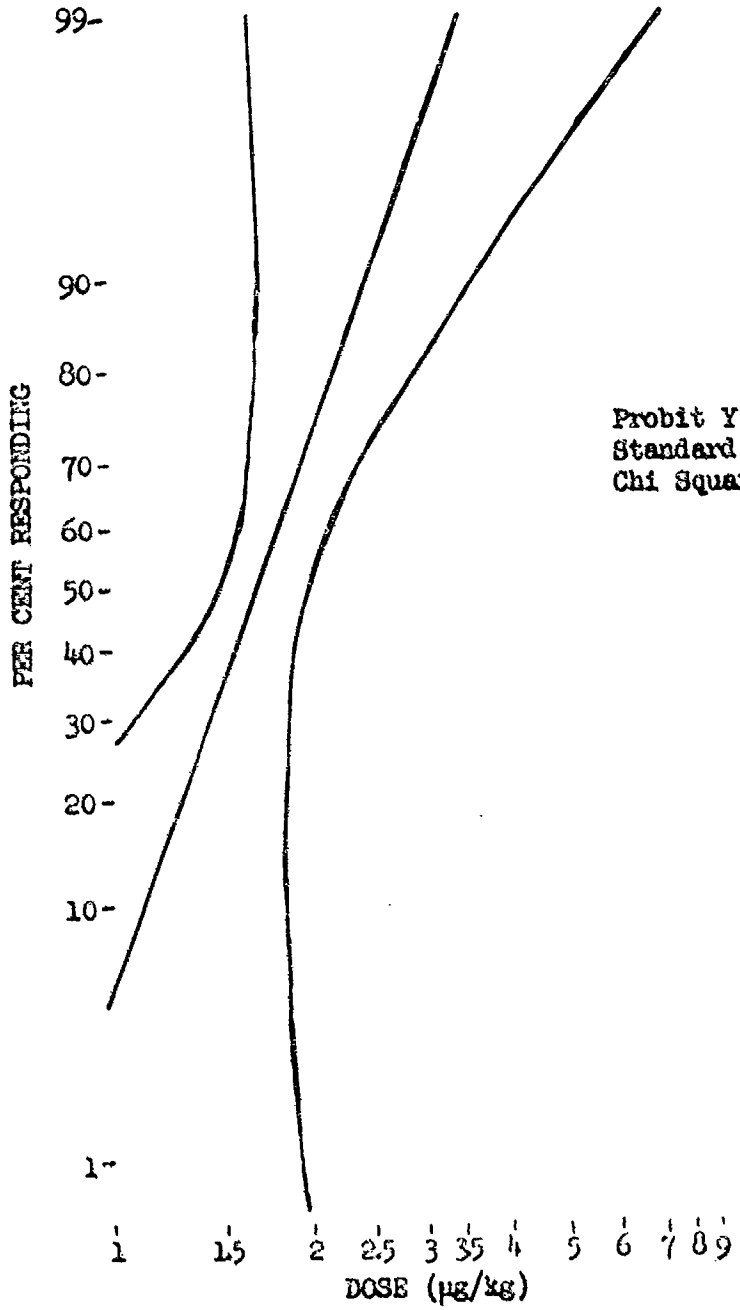
TABLE 1

EFFECTS OF A PRODUCTION MIXTURE OF ISOMERS 2 AND 4 OF EA 2233 ADMINISTERED INTRAVENOUSLY TO HUMANS (U)

Dose μg/kg	Subject No.	Orthostatic hypotension	Time of onset hr	Duration hr	Other symptoms*
0.5	1	None			
	2	None			
1.0	3	None			
	4	None			
1.25	5	None			
	6	None			
	7	None			
	8	+	3	1	
1.5	9	None			
	10	None			
	11	+	3	0.5	+
	12	+	1	7	+
1.75	13	None			
	14	None			
	15	+	1	5	+
	16	None			
	17	None			
	18	+	2.5	0.5	+
	19	None			
	20	None			
2.0	21	None			
	22	None			
	23	None			
	24	+	0.5	28	+
	25	+	2	6	
	26	+	4	0.5	
	**27	+	0.75	12	+
	**28	+	3.5	1	+
2.25	**29	+	3	3	+
	30	+	2.5	12	+
	31	+	4.5	7	+
	32	None			
2.55	**33	+	1	21	+
	**34	+	2.5	16	+
	35	+	6.5	4	+
	**36	+	4	23	+
	37	+	1.5	16	+
	38	+	1.5	9	+

*(U) Lightheadedness, nausea, and pallor.

**(U) These subjects were treated with methoxamine or phenylephrine during the course of their hypotension.

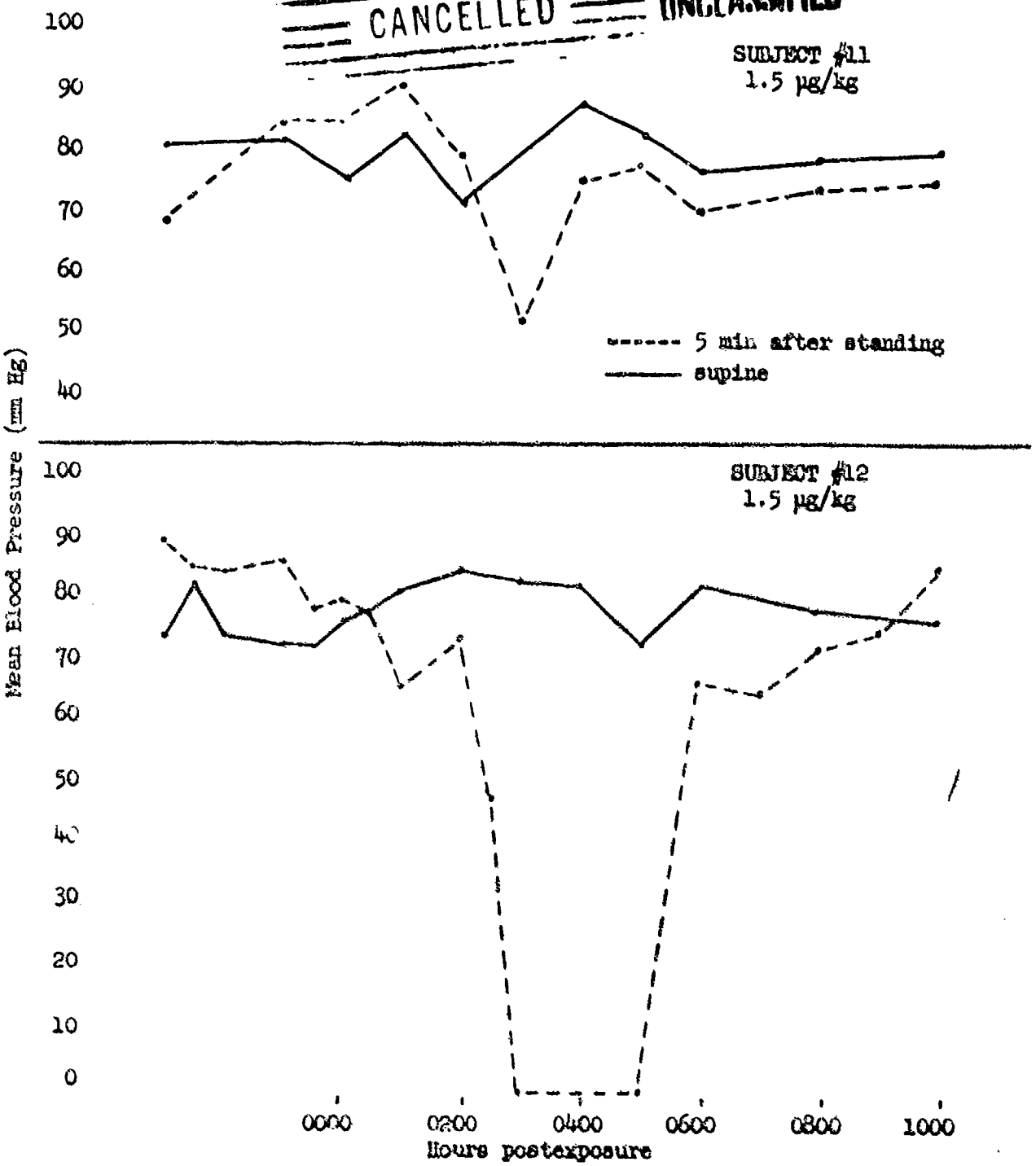


Probit Y = 3.15 + 7.44 Log X
Standard error of slope = 2.56
Chi Square = 4.43

(C)

FIGURE 1

PROBIT REGRESSION ANALYSIS AND 95 PER CENT CONFIDENCE LIMITS FOR A POSITIVE RESPONSE TO A PRODUCTION MIXTURE OF ISOMERS 2 AND 4 OF BA 2233 (U)



(C)

FIGURE 2

MINIMAL AND SEVERE RESPONSES TO ISOMERS 2 AND 4, EA 2233 (U)

(C) Other symptoms and signs that occurred with EA 2233 were lethargy, dry mouth and skin, and mild conjunctivitis.* These occurred in almost all the subjects including those given only 0.5 µg/kg. Each of these symptoms occurred about 1 hr after the drug was injected and usually persisted 24 hr. None were severe. The men were easily arousable and could function well. None of the men complained unduly about their dry mouth, which was temporarily relieved by water. None had visual difficulties. All the men with an orthostatic hypotensive response associated with symptoms also exhibited a strange greenish pallor after standing for 5 min. In a few men this persisted after they lay down.

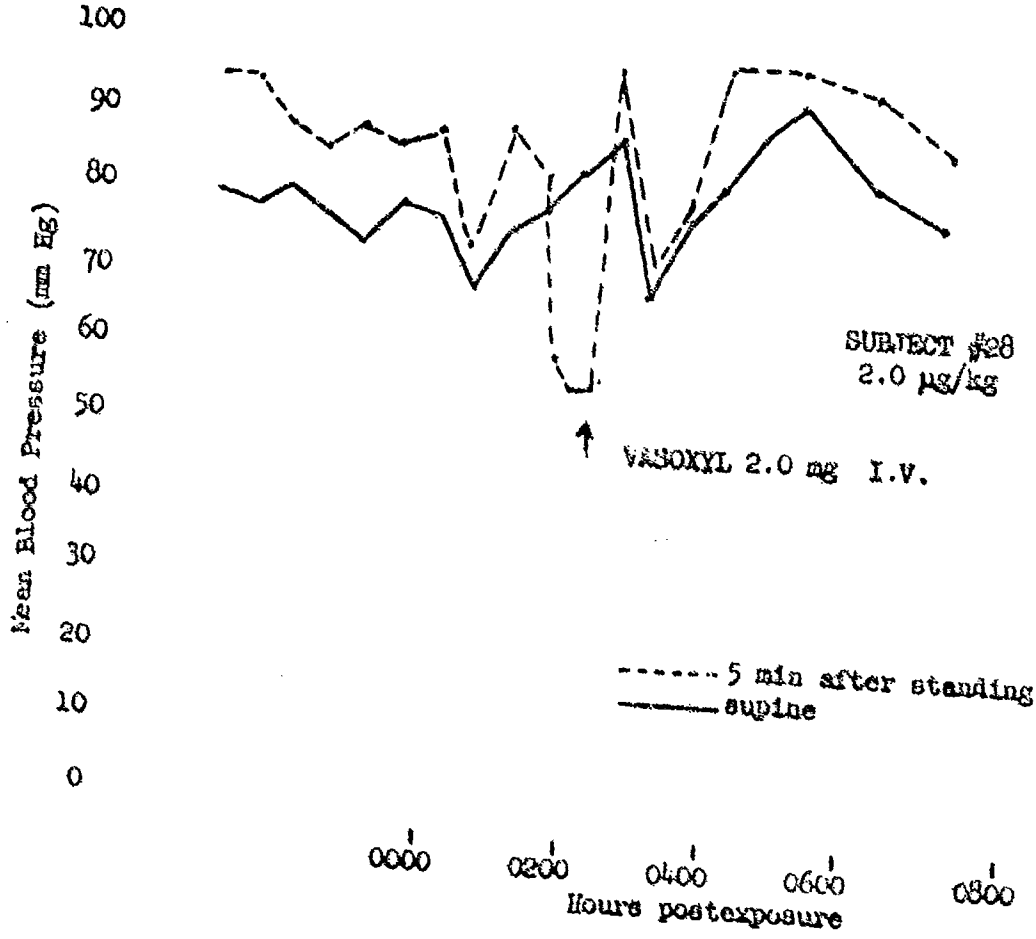
(U) Two individuals had urinary retention of approximately 750 ml, but catheterization was not required. Two men had transient flattening and inversion of their T waves in Lead II of the EKG, which was not associated with cardiac symptoms. There was no significant change in the Number Facility test scores, pupil size, or temperature of any of the men tested. In the present series, there was no change in laboratory values at 24 hr, 48 hr, and 10 days post-injection for any of the men.

(C) Six men were given sympathomimetic drugs during an orthostatic hypotensive response to determine the efficacy of possible therapeutic procedures. The drugs used were methoxamine HCl (Vasoxyl® HCl), 2 to 5 mg intravenously, and phenylephrine HCl (Neo-Synephrine® HCl), 0.5 mg intravenously. Each time that these drugs were administered there was a rapid (2 to 5 min) remission of the orthostatic hypotension and all symptoms (figure 3). Concomitantly, there was a mild rise in mean blood pressure in the supine position. The beneficial effects of methoxamine, 5 mg intravenously, can be expected to persist 45 min. Phenylephrine, 0.5 mg intravenously, also temporarily reversed the hypotension caused by EA 2233. Its effect, however, was not as prolonged as that of methoxamine. None of the subjects developed hypotension while in the supine position. Higher doses of EA 2233 or an accidental exposure to this drug, however, might cause severe supine hypotension. In these cases, sympathomimetic compounds should be effective antidotes.

IV. (C) DISCUSSION.

(U) Orthostatic hypotension is sometimes a normal psychogenic response. Usually, mean blood pressure rises slightly when man assumes the standing position. However, this may be variable in the same subject. Subject No. 39, for example, received 1.5 ml saline intravenously. His mean supine blood pressure was 93 mm Hg; after he stood for 5 min, his mean blood pressure fell to 43 mm Hg (figure 4). Associated with this decrease were symptoms of lightheadedness and faintness. On the next reading (after standing 5 min) a half hour later, his mean blood pressure was 85 mm Hg and he felt well. Eight hours after injection, his mean blood pressure (after standing

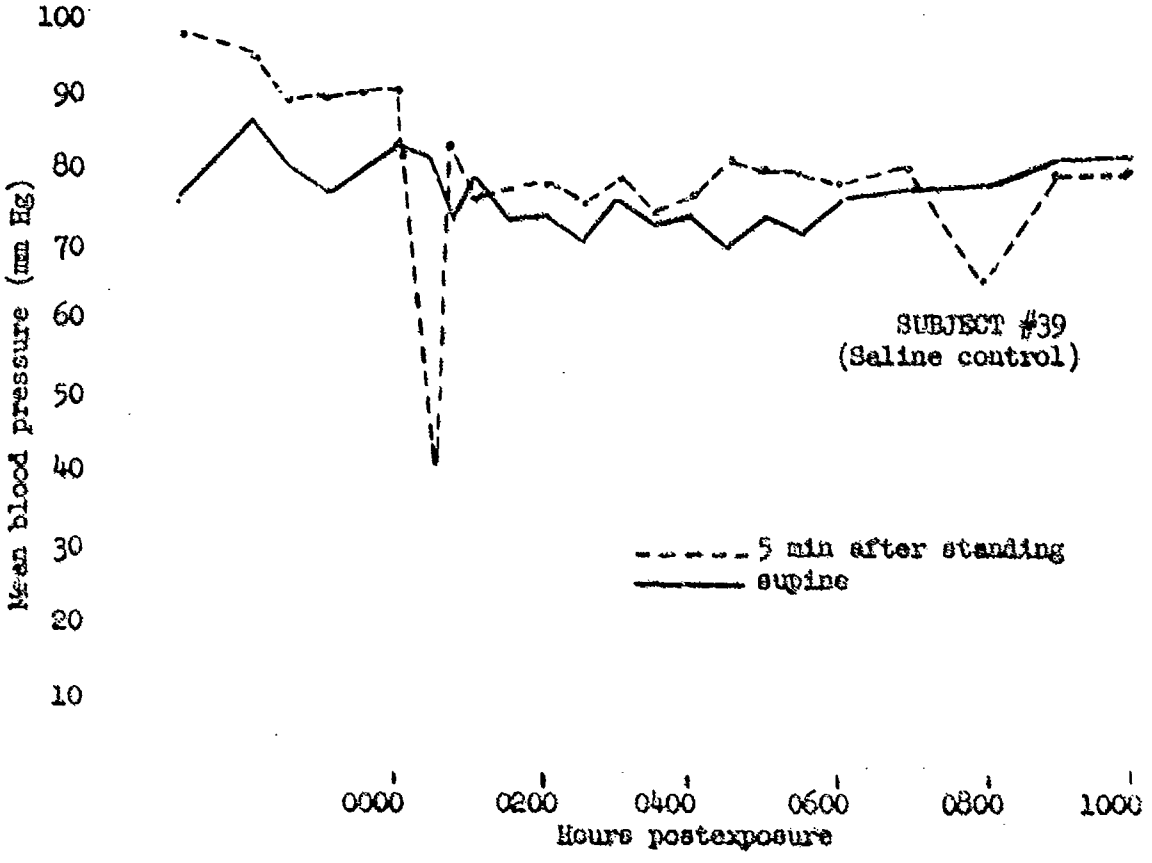
*These symptoms are in addition to those listed in table 1, i.e., nausea, lightheadedness, and pallor.



(C)

FIGURE 3

EFFECT OF VASOXYL ON ORTHOSTATIC HYPOTENSION RESULTING FROM ISOMERS 2 AND 4, EA 2233 (U)



(U)

FIGURE 4

PSYCHOGENIC RESPONSE TO INTRAVENOUS SALINE (U)

5 min) again decreased to 67 mm Hg, but he had no symptoms.

(C) In an attempt to eliminate psychogenic responses due to normal variations, at least 4 control blood pressure series (as described under Methods) were performed before the injection of EA 2233. Also, a decrease in standing blood pressure had to occur on two successive occasions for the subject's response to be considered positive.

(C) The major symptoms and signs produced by EA 2233 in these studies occurred while the subject was standing. As soon as he would lie down, the blood pressure would return to normal and all symptoms would rapidly disappear. All the subjects were capable of performing normally when they were in the supine or sitting position. None had a significant decrease in Number Facility scores when measured while the subjects were in the supine or sitting position.

V. (C) CONCLUSIONS.

From this study of a production-line mixture of isomers 2 and 4 of EA 2233 administered intravenously to humans, it is concluded that the MED50 for orthostatic hypotension (defined as a difference of at least 10 mm Hg between supine and standing mean blood pressure occurring on 2 successive readings 30 min apart) is 1.8 μg/kg (1.51-2.07). There was no significant decrement in mental function as measured by the Number Facility test.

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13. ABSTRACT

(C) A production-line mixture of Isomers 2 and 4 of EA 2233 was administered intravenously to 38 medical volunteers. Nineteen men experienced orthostatic hypotension. Probit analysis by the method of Bliss indicated that the MED50 for orthostatic hypotension was 1.8 µg/kg. Vasoxyl (methoxamine) and Neosynephrine (phenylephrine) were used to treat the orthostatic hypotension and found to be effective.

14. (U) KEYWORDS.

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Humans
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Treatment
Sympathomimetic amines

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